## Hybrid pore formation by directed insertion of alpha hemolysin into solid-state nanopores

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## **Supplementary material**



**Figure S1 Conductance histogram.** A histogram of conductance measurements (relative to the baseline level) for the SS-nanopore in Fig. 2a prior to and during  $\alpha$ HL insertion. The first peak (gray) represents baseline conductance of the initial nanopore. The second and third peaks (black) represent dsDNA translocation through the SS-pore, indicating attempted and true translocations, as described by Ref. 8 in the main text. The final peak (red) is a level of conductance unique to material with  $\alpha$ HL attached and represents formation of a hybrid nanopore structure.

## SUPPLEMENTARY INFORMATION



Figure S2 Additional examples of protein capture events. Four traces of nanopore conductance measured during  $\alpha$ HL insertion. The three-phase events are colored as in Fig. 2a in the main text. These traces are from four different SS-nanopores and are recorded at (a) -150 mV, (b) -200 mV, (c) -500 mV, and (d) -600 mV applied to the *cis* chamber, respectively.



**Figure S3 Measured nanopore conductance before and after aHL insertion.** Measured conductance of 21 SS-nanopores both before (gray) and after (red)  $\alpha$ HL is inserted. SS-pores of slightly different sizes yield a final measured conductance (1.0 ± .5 nS) in good agreement with native  $\alpha$ HL in a lipid membrane (1.0 nS). We observe no correlation between the conductance value before and after insertion.



Figure S4 Hybrid nanopore residence statistics. Duration of  $\alpha$ HL residence inside the SS-nanopore for the 21 devices presented here. 'Minutes' signifies residence time of 1-60 minutes, 'hours' of 1-24 hours and 'days' of >24 hours. Nearly half remain viable for periods long enough to be used in further measurements.



Figure S5 Current noise analysis for three typical hybrid nanopores. Traces show power spectral density of the current noise both before (black) and after (red) insertion of an  $\alpha$ HL protein into three typical pores, measured at (a) 600 mV, (b) 300 mV and (c) 400 mV. (a) is the capture shown in Fig. 2a of the main text. The solid lines are spline fits to the displayed data to clarify the trends.

## SUPPLEMENTARY INFORMATION



Figure S6 Additional example of poly(dA)<sub>100</sub> translocation through a hybrid pore. (a) Recorded current trace through another hybrid nanopore (V = -500 mV applied to the *cis* side), showing the baseline conductance directly after insertion (left) and events upon addition of poly(dA)<sub>100</sub> (middle). At right is an expanded view of a typical event (red line indicates the fit). (b)  $\Delta G$  vs dwell time scatter plot (171 events) and corresponding histograms for this additional hybrid nanopore. The bimodal distribution of  $\Delta G$  has centers at 0.41 and 0.72 nS, respectively while the distribution of dwell times is centered at 110 µs. This example yields a slightly lower dwell time than the example in the text due to the larger applied voltage.



**Figure S7 Electrical breakdown of a solid-state membrane.** Measured current across an undrilled SS-membrane (20 nm thick SiN) under increasing applied voltage. Failure occurs at 2.88 V. The solid line is a linear fit to the data after the break.